Interior Spruce Pollen Management Manual

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Interior Spruce Pollen Management Manual

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1 INTRODUCTION

Coniferous pollen is a readily mobile gamete making it a powerful tool for manipulating seed production in tree breeding and seed orchard programs. To successfully meet specific objectives, however, pollen must be carefully managed. This will involve the processes of collection, extraction, storage and reapplication. The extent to which controlled crossing or supplementally applied pollen results in seed yields of known paternal parentage will depend to a large degree on our ability to maintain pollen viability and vigour over its handling period. Of equal importance in optimizing seed yields and maximizing genetic quality is synchronizing pollination with optimal seed cone receptivity.

This manual is directed primarily towards managers of interior spruce soil-based seed orchards within British Columbia. It also contains information that may be useful for managing pollen within the breeding and container seed orchard programs as well. The container seed orchard program has a separate pollen manual for its specific needs. This manual could also be used for other developing tree improvement programs since the techniques described are not necessarily species specific.

Most of the recommendations for implementing pollen management procedures in interior spruce seed orchard operations are based on data collected from field experiments. There are also several other published sources of useful information, the most pertinent of which are listed in Section 5, "Relevant References".

Since the interior spruce seed orchard program does not distinguish between white, Engelmann or their hybrids, there has been no attempt to isolate species for either field or laboratory testing. For the present, specific data and procedures discussed should be equally valid for either species or their hybrids.

Where possible, specific recommendations for handling pollen will be given. However, certain procedures remain untested, or are still being evaluated. In these cases "best-guess" procedures will be offered and the user is encouraged tc monitor the response of their treatments in order to better appreciate the extent of variability and the limits of confidence. This is particularly true for extrapolating the results of *in vitro* viability assays into fertility potential or assuming supplementally applied pollen will always compete successfully.

First, this manual will offer a brief rationale for incorporating pollen management procedures into an intensive seed orchard program. Then, specific guidelines for managing interior spruce pollen are covered under the following major headings:

1. pollen handling (including collection, extraction and storage);
2. pollen testing (viability assays);
3. pollen application; and
4. pollen monitoring.

2 INTENSIVE SEED ORCHARD MANAGEMENT

Supplying abundant quantities of seed and maximizing genetic gain potential are two important objectives of all seed orchard programs. Our experience in soil-based orchards suggests that abundant seed production is possible within 6-8 years after establishment. However, in unmanaged orchards which rely solely on wind pollination, optimal yields or genetic gain potential are seldom realized. The extent to which orchard seed reflects both the genetic potential and broad diversity of orchard parent trees is termed genetic efficiency (Adams 1983; El-Kassaby *et al.* 1984).

To obtain full genetic efficiency from orchard seed, certain criteria must be satisfied first (Eriksson *et al.* 1973; Woessner and Franklin 1973):

1. all parent trees contribute equally to seed production;
2. contamination from extraneous pollen is negligible; and
3. inbreeding within the breeding population is not significant.

For interior spruce orchards located away from indigenous spruce stands, local pollen contamination is not expected to have a serious effect on genetic efficiency. Whether infiltration of pollen from other orchards will have a negative effect on genetic efficiency has yet to be determined.

Inbreeding could have a negative impact on both seed yields and genetic efficiency, but the extent to which inbreeding occurs will depend on the overall relatedness of orchard clones. Selfing is always possible but it is not expected to be significant. Self-only pollen can produce about 25% of expected outcross yields (10-20 seeds per cone) but where outcross pollen is freely available, then less than 10% would be attributed to self pollen (G. Kiss, B.C. Ministry of Forest, pers. comm.).

Parental imbalance, however, is expected to have a serious effect on genetic efficiency. Non-synchronous receptivity and unequal fecundity among seed orchard parent trees (El-Kassaby *et al.* 1984) occur regularly and may be the single most important factor affecting the realization of full genetic gain potential in interior spruce seed orchards.

2.1 Orchard Dynamics

In the initial phase of orchard development, considerable variation in flowering fecundity and non-synchrony between clone receptivity periods are expected. Assuming sufficient within orchard pollen is available, then discrete breeding populations will occur. Although it is not known how this will affect genetic gain, we assume its effect will be negative and genetic diversity will be lower.

Perhaps our most important seed orchard management objective (especially in advanced generation orchards) will be balancing production and facilitating random mating among all parents. This stresses the importance of monitoring flowering and phenological behaviour to determine which clones may require cone-induction treatment or application of pollen management techniques to improve genetic efficiency. Monitoring will become a repeated theme throughout this report and its importance in the implementation of an intensive seed orchard program cannot be over stressed.

Monitoring within seed orchards will include cone crop surveys (scoring relative crop intensity by clone), seed cone receptivity periods, and pollen cone shedding dates (see Sections 3.11, 3.31 and 3.32) as well as the monitoring of daily pollen cloud densities (Section 3.4). This information will become the basis for estimating within orchard variation of pollen quantity and parental composition (including contaminating sources) available to any particular clone at any time. Once this information is compiled, it forms a yearly record of orchard dynamics which can be used for developing orchard management practices to protect genetic efficiency of subsequent years crops.

2.2 Optimizing Genetic Efficiency

Among the techniques available to protect orchard efficiency is overhead irrigation and supplemental pollination. Cooling the orchard with a mist spray is used in coastal Douglas-fir orchards to slow reproductive development relative to pollen development of local, contaminating sources (see Fashler and El-Kassaby 1987). However, since contamination of interior orchards is not currently a significant concern, this technique will not likely be employed. A more widely used technique for protecting orchard efficiency is supplemental pollination.

Supplemental pollination, supplemental-mass-pollination (SMP), or boosting are all terms used to describe the process or application of pollen to exposed, receptive seed-cone buds. This technique has been used to improve seed yields (Bridgewater and Bramlett 1982; Webber 1987; and, El-Kassaby and Reynolds 1989); introduce specific genotypes into the seed orchard (Woessner and Franklin 1973; Hadders 1984); reduce self-fertilization and contamination (El-Kassaby and Ritland 1986; Wheeler and Jech 1986); and improve parental balance (El-Kassaby and Reynolds 1989).

Seed yields of interior spruce can be increased by boosting pollen supply (see Table 3, Section 3.342) but the response will depend on the extent of competing pollen cloud density. A more difficult task, however, will be determining boosting effects on improving parental balance and reducing selfing or contamination.

Actual technique employed for applying pollen supplementally will depend on specific objectives (i.e., whether to achieve specific crosses or general orchard boosting) and, to a certain extent, on flowering intensity and receptivity periods of individual clones (again emphasizing the importance of monitoring both intensity and receptivity periods for all parent trees).

Also, variation in the orchard pollen cloud density will determine, to a large extent, how well supplementally applied pollen will compete with the existing orchard pollen cloud. Certainly, application technique (timing, amount and applicator type) interacts with competing pollen cloud density and will play an important role in determining success of supplemental pollination (Webber 1987). Finally, but not least, is pollen vigour. Considerable effort will be expended by orchard operators to complete a pollen boosting program. It would, therefore, be of little value to apply pollen of poor vigour or fertility potential. The importance of using and maintaining (storing) pollen of highest possible fertility potential will also be emphasized throughout this manual.

3 POLLEN MANAGEMENT

3.1 Pollen Handling

Success in handling, storage and post-storage application of pollen depends largely on its initial vigour. Little can be done to affect pollen development and maturation in soil-based seed orchards. It is possible, however, to assess yearly variation in both pollen yields and viability to appreciate various environmental factors (especially temperature) on pollen development. It would be a mistake to assume pollen quality will be the same each year.

The environmental effects on pollen or microspore initiation and development are most critical during the initial stages of meiosis in the post-dormant bud. This process occurs within the pollen mother cells and leads to the production of individual pollen grains (see Owens and Blake 1985). Meiotic activity in the post-dormant bud may begin in response to temperature and can be a very sensitive stage of development (see Owens and Blake 1985 and references cited therein).

Environmental effects on pollen development and quality are not easily determined and they are likely to vary with orchard site and year. Orchard managers are encouraged to monitor at least temperature (and, if possible, humidity levels and wind direction for the pollination period) during post dormancy development of the pollen bud. If abnormal development of pollen is suspected, it is possible to assess pollen viability using laboratory testing procedures. Incorporating *in vitro* viability techniques into operational seed orchard management and using them to assess potential pollen fertility will be discussed in Section 3.2.

Heat sum values (total hours, days, etc. above a specified temperature level), can be particularly useful for estimating seed cone receptivity and pollen shedding dates when environmental conditions are apt to vary considerably from year to year. Calculating heat sum values can be routinely determined, especially if environmental data loggers are employed. They offer great potential for quantifying receptivity periods and shedding dates between clones as well as quantifying potential contamination dates (where applicable).

It is likely that the number of degree hours above either 5°C or 10°C will be useful temperature thresholds, but this must be evaluated over a series of years. Environmental monitoring is an important adjunct to all orchard management programs including assessing temperature effects on seed cone abortion and development.

3.11 Pollen collection

To maximize both the quantity and quality of pollen yields, cone buds should be collected as close to anthesis (shedding) as possible. Figure 1A shows four of five stages of interior spruce pollen-cone bud development which can be readily identified. Picking should occur at Stage 4.

Stage 1 is the dormant bud. At this stage it is virtually impossible to distinguish between male, female or vegetative buds without dissection. Pollen-cone buds are normally located in the lower crown and can occur all along the stem. In the upper regions of the crown, male buds can be located at the base of the stem. Even then, it is not easy to identify bud types.

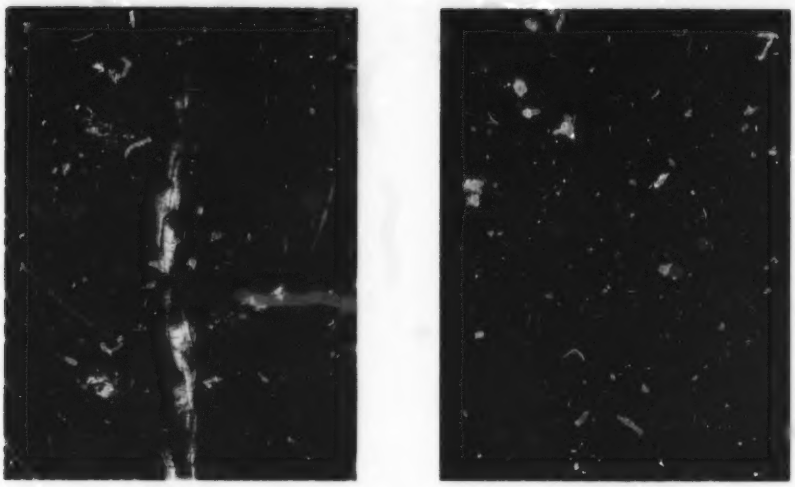
Stage 2 is the first stage of development that is readily recognized. Stage 2 buds are characterized by a slow but steady increase in bud girth (pollen grains are forming and developing) over a 4 or 5 week period. In the southern Interior, pollen buds normally begin to break dormancy in late March, but one can expect considerable variation between sites.

Seed-cone buds also begin to swell during this period and it may be possible to distinguish them from male and vegetative buds by their position in the crown and on the stem. Normally, seed-cone buds are more slender in appearance and occur primarily in terminal positions, although in heavy crop years seed-cone buds can occur all along the stem. The only certain way to distinguish between bud types, however, is either to dissect them or to wait for bud burst.

Stage 3 of pollen cone development is bud burst (BB). About 4 weeks after bud swelling begins, the elongating pollen cones burst through their bud scales, exposing the red tip of the microsporophylls. Microsporophylls are the bract-like structures of the cone that bear the pollen sacs, the microsporangia (see Figures 1A and 1B). The pollen bud axis continues to elongate and the microsporangia become more visible. As the pollen cone approaches shedding, the microsporangia enlarge and become readily distinguishable from adjacent microsporangia. Characteristics of late stage 3 buds are:

1. individual microsporangia are easily distinguishable but still tightly packed; and
2. pollen buds express a clear, watery liquid when squeezed between the fingers.

Although stage 3 pollen buds may appear mature, they are not if the expressed liquid is clear and watery. If pollen is harvested at this stage, yields will be lower and drying will take longer. Although this stage is easily determined at the beginning, it is difficult to know exactly when it ends. However, elongation of the cone axis is an indication that stage 4 is approaching. Stage 4 (shedding) can be reached very quickly under warm temperatures and low humidities. Pollen buds at this stage of development must be monitored frequently.



STAGE 2

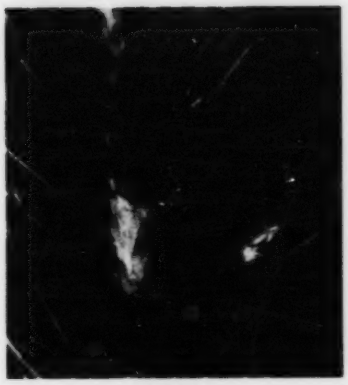
STAGE 3 (BB)

STAGE 4

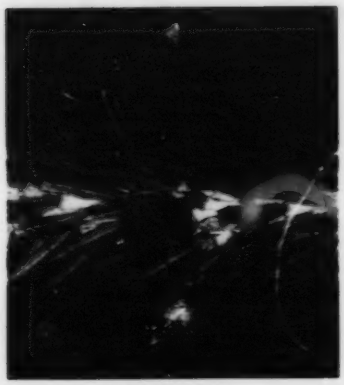
STAGE 5

FIGURE 1A. Four stages of pollen cone development in interior spruce.

FIGURE 1B. Variation in harvesting stage of interior spruce pollen-cone buds.



STAGE 2

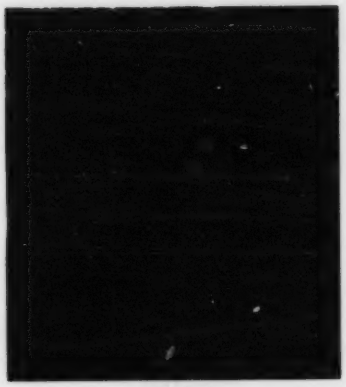


STAGE 3 (BB)

STAGE 4

EARLY ..... MID ..... LATE

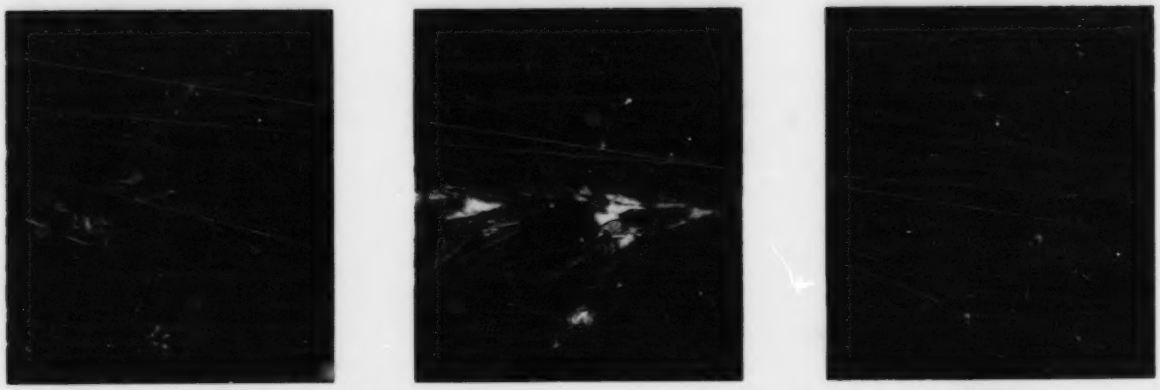
EXAMPLES OF STAGE 4 RECEPTIVITY



STAGE 5

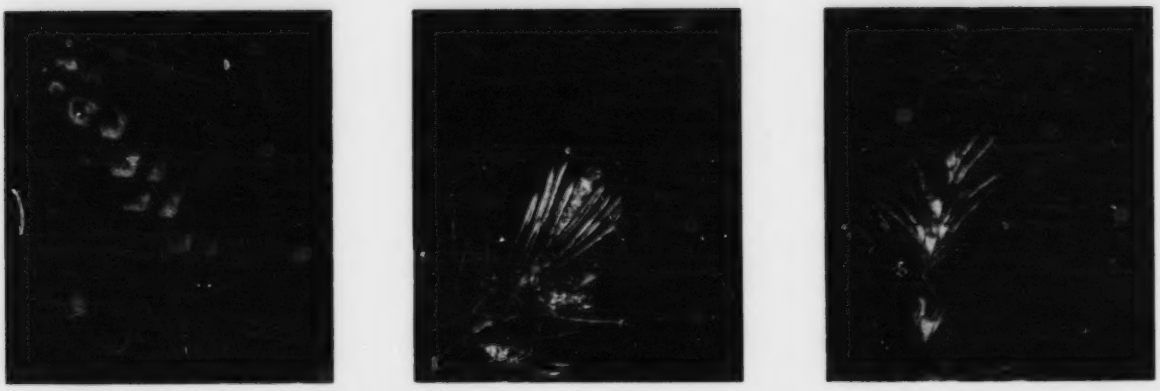
EXAMPLES OF STAGE 1 TO STAGE 4

FIGURE 2A. Various phenological stages of interior spruce seed-cone bud receptivity.

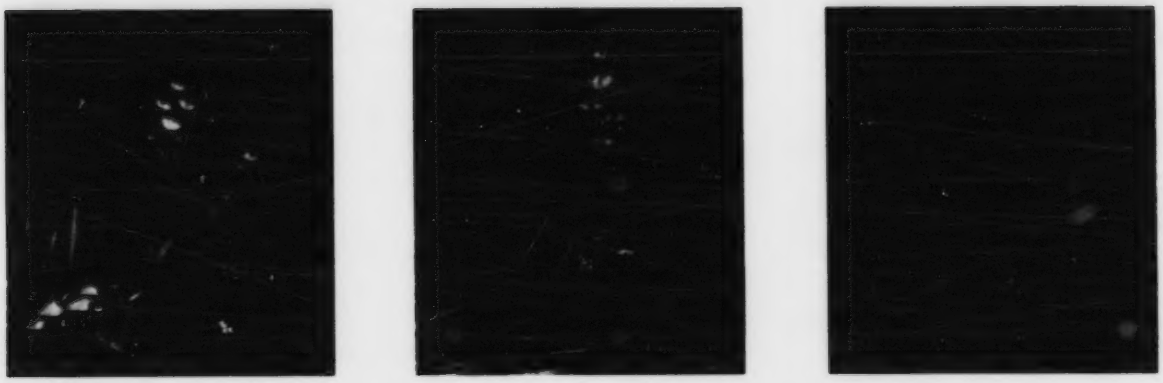


STAGE 3 BUD BURST

STAGE 4



STAGE 4 RECEPTIVITY



STAGE 5 CONE CLOSURE

FIGURE 2B. Examples of three phenological stages of interior spruce seed-cone bud receptivity.

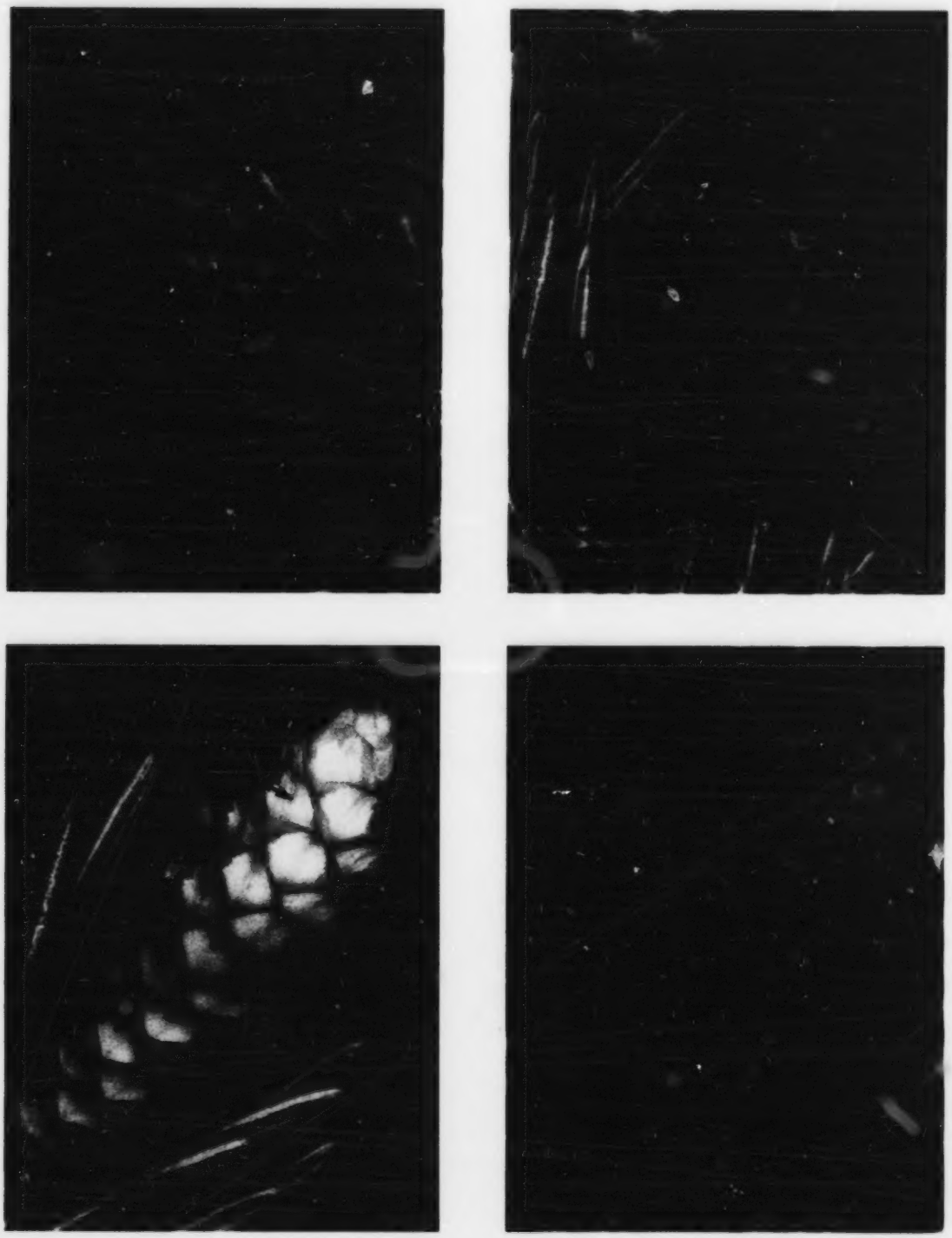


FIGURE 2C. Four examples of the optimal time to pollinate interior spruce seed-cone buds (Stage 4 receptivity).

Stage 4 is the mature pollen-cone bud which occurs just before shedding. At this stage, microsporangia become visibly separated (see Figure 1B) and the moisture that can be expressed from the bud is yellowish and more viscous. Another characteristic of stage 4 buds, resulting from elongation of the microsporangia along the cone axis, is that the entire pollen bud becomes loose and, when it is compressed between the fingers and then released, the bud more or less springs back to its original shape. For maximum pollen yields and maturation, buds should be picked at this stage.

Under operational conditions, however, it may not be possible to pick all buds at the optimal stage. During warm weather when humidity levels are low, pollen development and shedding can occur rapidly. Furthermore, development on the south side of the tree can be one to several days more advanced than on the north side. Thus, it is likely that various stages of pollen development will be included in the harvested crop. What effect this will have on yields and potential fertility is being considered in future pollen extraction and drying trials. Where possible, pick only stage 4 buds.

Figure 1B shows various examples of stage 4 buds. In all cases the buds have elongated; the microsporophylls are showing signs of loosening up; individual microsporangia are more readily visible; and, perhaps most important, the expressed liquid is yellowish and viscous.

Stage 5 buds are completely shed and the remaining spent pollen cone can remain on the tree for a few days or even weeks.

3.12 Pollen extraction

Harvesting pollen-cone buds, in general, can be a tedious task and there are few simple procedures. However, for spruce, hand picking is relatively easy because the buds are quite large. Harvested pollen buds should be moved to the extractory as soon as possible and not be left to heat up (i.e., in the field or the back of the truck). Washing pollen buds is a standard practice used by tree breeders for the removal of undesirable, extraneous pollen that may be adhering to the buds. This should not be a real concern in seed orchards, and washing of pollen buds prior to extraction is not recommended.

For interior spruce, we have little information on the post-harvest storage of pollen-cone buds. For Douglas-fir, it is possible to store pollen-cone buds up to 2 weeks at 5°C without affecting either yields or viability. Interior spruce buds have been stored for 10 days at 5°C without affecting yields or *in vitro* viability. If it is necessary to store interior spruce pollen-cone buds, then place them in paper bags (not plastic) and maintain them between 2-5°C, but not for longer than 10 days.

Standard drying practice for all conifer pollen-cone buds generally uses temperatures between 20-30°C and relative humidity values less than 40% (G. Kiss, B.C. Ministry of Forests, Kalamalka Research Station) has been successfully extracting (and storing) pollen for many years. Here, small lots (100 mL or less) of spruce pollen-cone buds are extracted between 25-30°C for 12-24 hours (humidity levels were not monitored). More recently, experience gained from a prototype research dryer suggests bulk quantities of pollen buds (21 L) to can be dried to storage moisture contents within 24-48 hours (depending on the initial %MC of the buds). A larger operational dryer has been recently completed and will be employed to dry even larger quantities (100-150 L) of pollen-cone buds. The main advantage of these dryers is that they can maintain constant drying conditions (especially humidity) over changing atmospheric conditions and thereby make drying schedules (and, hopefully, pollen quality) more consistent.

Drying at 30°C with conditioned air that is below 20% RH appears to effectively dry the pollen buds in a reasonable time (less than 48 hours). The effects of various temperature and humidity levels on extraction times, *in vitro* viability and storability of spruce are being evaluated. Until further data is available, recommended drying limits for the operational extraction of spruce buds should not exceed 30°C (lower is preferable) or allow humidity levels to drop below 20% RH. Although there is no evidence suggesting that rapid drying is detrimental to pollen quality, any extreme conditions should be avoided.

During harvesting, drying, and extraction of the pollen, care should be taken to protect the pollen from exposures to high humidity levels as well. Under no conditions (except perhaps the first few hours of

pollen-cone bud drying) should either the pollen buds (or pollen itself) be exposed to RH values >70% and/or temperatures >30°C for more than 10-12 hours. This is especially important during the later stages of drying and post-drying handling. Otherwise, rapid loss of viability and fertility could occur.

3.13 Pollen storage

Factors affecting successful storage of pollen are moisture content, temperature and storage atmosphere. Table 1 summarizes the combined averages for three main treatment factors applied to interior spruce pollen stored for 1 and 3 years. Pollen lots (four) at four moisture contents (4, 8, 12, and 16% MC) were stored at two temperatures (+5°C and -25°C) under three different atmospheres (air, nitrogen and vacuum).

TABLE 1. Effect of storage moisture content, temperature, and atmosphere on filled seeds per cone (with standard errors) of interior spruce pollen stored for 1 and 3 years.

Treatment		Storage period	
		One year FSPC[a] ± se	Three years FSPC ± se
Temperature	+5°C	22.0 1.0	31.9 1.3
	-25°C	30.2 1.2	48.0 1.4
Moisture content	4%	29.6 1.8	53.7 1.8
	8%	28.2 1.6	50.4 2.0
	12%	28.4 1.6	46.2 1.9
	16%	19.1 1.5	11.1 1.1
Atmosphere	air	22.2 1.3	23.5 1.3
	nitrogen	30.6 1.5	47.1 1.7
	vacuum	26.4 1.4	49.7 1.8

[a] Filled seeds per cone ± standard error.

Heat-sealed aluminum foil packets were used to store pollen lots under an atmosphere of either air or nitrogen. Evacuated glass vials were used for storing pollen under vacuum. Previous trials indicated that container type (i.e., glass or foil laminated pouches) did not affect pollen fertility potential during storage.

As expected, storage atmosphere and temperature had important effects on seed yields. Pollen moisture content also affected yields but, for the range tested, a significant reduction in pollen fertility was observed only for pollen lots stored at a moisture content of 16%. This is in contrast to Douglas-fir pollen which clearly showed that pollen moisture contents above 10% could not be tolerated.

The effect of pollen moisture content, storage temperature, and atmosphere as interacting factors is summarized in Figure 3. Maximum seed yield values for interior spruce pollen stored 3 years occurred when pollen moisture content was 8%, the storage atmosphere did not contain air, and the storage temperature was - 25°C. Pollen stored in air at 5°C lost considerable fertility potential, especially for pollen moisture contents above 4%.

The results shown in Table 1 and Figure 3 are average values for four pollen lots applied to each of four clones (seed-cone parents). As one might expect, variation in seed yields was observed for both the lots and clones (used as seed-cone parents).

Figure 4 shows the mean yield values (over all factors) of the four pollen lots (stored for 1 and 3 years) on each of four separate seed-cone parents (not the same parents in each year). A clone-by-pollen lot interaction observed for seed yield is a normal occurrence and may account for some of the low yield values shown in Figure 4 (i.e., lot 2 on clone 1 for third year storage results). However, low filled seed per cone values (especially for clones 2 and 3 from the first year test) probably reflect pollinations at a less-than-optimum stage of receptivity. Refer to Section 3.32 for a more detailed discussion on the optimal time to pollinate interior spruce.

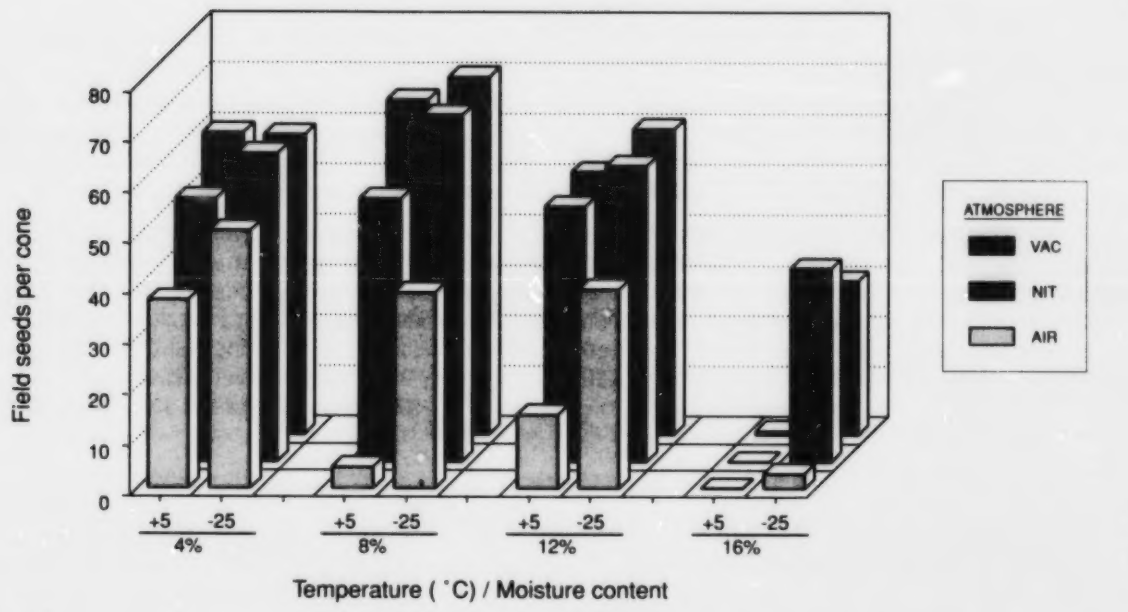


FIGURE 3. Effect on seed yield of storing interior spruce pollen at four moisture contents, two temperatures and three atmospheres for three years' duration.

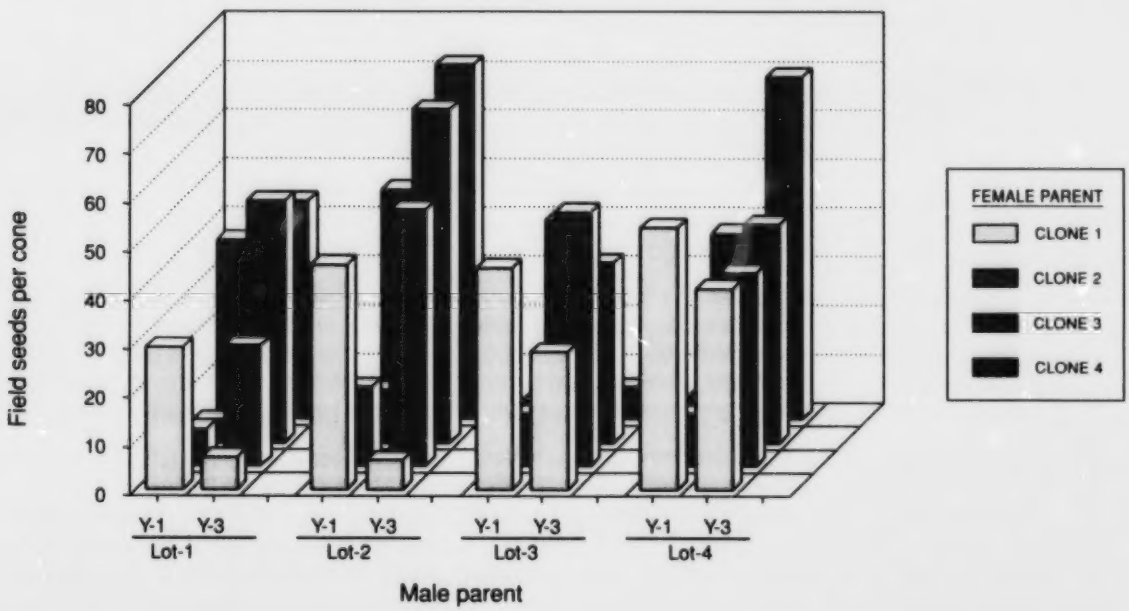


FIGURE 4. Effect on seed yield of pollinating four interior spruce clones (seed parents) with four separate pollen lots after one and three years' storage. Note the four clones (seed-parent clones) for each of the two storage periods are not necessarily the same.

3.14 Determination of pollen moisture content

Pollen moisture content is an important factor affecting long-term storage success. It can not be over-emphasized that moisture content must be determined before a particular lot is put into storage. It will also be important to monitor %MC from the pollen extraction unit to optimize drying schedules.

Moisture content is determined using the oven-dry technique and is based on the wet weight of pollen used. Routine %MC determination procedures use two replicates of 500 mg of pollen heated in an oven at 85°C for 4 hours. If pollen is scarce, then less weight can be used (200 mg) and only one replicate need be tested. However, the error of determination will be higher!

Other drying conditions (e.g., 100°C for one hour) have been used as well, but using temperatures near 100°C can over estimate %MC because chemical constituents other than water can also boil off. It is also possible to determine moisture content using infrared-heating balances. Moisture determination balances are supplied by several companies (e.g., Ohaus, Mettler, Sartorius) and offer a rapid technique for estimating %MC. They do not, however, have the precision for determining %MC in small quantities (500 mg) and are relatively expensive ($2000-3000). They will provide quick estimates of %MC where precision is not important. If doubts about actual moisture content occur, use the gravimetric technique described below:

1. Record the weight of the pollen container (Wt_c).
2. Add 500 mg of wet pollen and record the weight (Wt_w).
3. Heat at 85°C for 4 hours.
4. Cool in a desiccator to room temperature.
5. Record the weight of the container plus dry pollen (Wt_d).
6. Calculate %MC as follows:

$$\%MC = \{[(Wt_w - Wt_c) - (Wt_d - Wt_c)] / (Wt_w - Wt_c)\} \times 100\%$$

3.15 Pollen storage conditions

Storing pollen at 6-8% MC at freezer temperature (-25°C) should not be difficult. However, storing pollen under an atmosphere other than air (e.g., nitrogen) may pose some technique problems. Pollen storage under vacuum was significantly better than under air only (Table 1) and until further storage data are available, storage conditions for interior spruce pollen will be defined as:

- Moisture content — 6-8% MC
- Storage atmosphere — vacuum
- Storage temperature — -25°C

When packaging this pollen for storage, a commercially available home vacuum-sealing unit was used. They are relatively inexpensive, easy to use, and sealing bags are readily available. If at all possible, vacuum sealing should be considered. If this is not possible, then make certain your pollen is in the 6-8% MC range, place it in an air-tight container, and try to fill the container so little, if any, air is left. Store the container in a freezer that is rarely used and capable of holding at least a steady -25°C.

3.2 Pollen Testing

Associated with all stages of pollen handling is the risk of affecting pollen viability and vigour. Considerable effort has been given to developing *in vitro* (in the laboratory) viability tests. It is intended that these procedures will indicate a pollen lot's viability (whether the pollen is alive or dead), its vigour (indicated by the rate of pollen tube growth) and its fertility potential (actual ability to carry out fertilization and form a seed).

In vitro viability tests have been developed primarily to estimate the effects of storage on pollen fertility potential. However, it is important to be able to estimate fertility potential of pollen at all stages of handling. This will be particularly important when orchard managers begin to formulate specific pollen poly-mixes to manipulate genetic composition of production seed.

3.21 Viability procedures

Currently, three techniques to estimate *in vitro* viability are being used:

1. oxygen uptake (a measure of respiration under controlled conditions);
2. electrical conductivity (a measure of pollen electrolytes leached into an aqueous solution); and
3. germination.

In general, respiration analyses has consistently produced the best estimates of fertility potential but may be technically more demanding to incorporate into operational procedures (costs are high as well). Both conductivity and germination are relatively simple to perform and can be used to determine whether a particular pollen lot is alive or dead. Correlation analyses between *in vitro* assays and fertility are ongoing, and more precise estimates of fertility potential may be available in the future.

3.22 Pollen germination procedures

Germination protocol has developed out of several tested procedures including aqueous and solid (agar) media types. Preliminary results suggests a modified procedure used by G. Kiss (B.C. Ministry of Forests, Kalamalka Research Station) provides the best germination and tube growth response. The medium is a composition of 0.5% agar made with 10% Brewbaker's (Brewbaker and Kwack 1963) solution in 10% sucrose. The medium used by the spruce breeding program is a mixture of sucrose and agar only. An aqueous solution of 5% sucrose and 10% Brewbaker's solution has also been used with success. For simplicity, the aqueous solution would be easier to formulate and use, but scoring germination is more difficult (some grains float while others sink). The recommended germination procedure is as follows:

Aqueous Solution (optional):

1. Brewbaker's stock solution (100 mL)

Boric acid [H_3BO_3]	100 mg
Calcium nitrate [$Ca(NO_3)_2.4H_2O$]	300 mg
Magnesium sulphate [$MgSO_4.7H_2O$]	200 mg
Potassium nitrate [KNO_3]	100 mg

Dissolve all ingredients in de-ionized water and make to 100 mL.

2. Brewbaker's working solution in 10% sucrose (100 mL)

   Add 10 g sucrose and 10 mL of Brewbakers's stock solution and make to 100 mL with de-ionized water.

Agar Medium (recommended):

3. Add 0.5 g agar (there are numerous types of agar on the market and almost any purified type will do) to the 100 mL Brewbaker's working solution in 10% sucrose. Gently heat the solution to boiling and cool until warm but still liquid. Pour about 3 or 4 mL of the solution into clean (preferably sterilized) 35-mm petri dishes and cover (prevent contaminating the surface of the agar). Cool the agar to room temperature. It is now ready for adding the pollen.

Pollen Rehydration:

4. Pollen that has been processed for handling or storage is normally in a dehydrated state. Although dehydration does not seem to affect pollen fertility potential (see Figure 9) it does affect its response to *in vitro* viability assays. As a routine procedure, all pollen lots are hydrated before testing. A small amount (30 mg) of pollen to be tested is sprinkled in an open (i.e., lid off) 35-mm petri dish and placed over water (we use a plastic ice-cream bucket with a tight-fitting lid, and a screen supported over water). The pollen is hydrated for 4 hours at 25°C. **Do not hydrate for longer periods!** Pollen viability may deteriorate with longer exposure periods.

Germination:

5. About 10 mg of the hydrated pollen is sprinkled (evenly, to avoid clumps, over the agar surface) and the lid is replaced. Also try puffing a small quantity of pollen in the air and pass the petri dish through it. The small petri dishes are then placed into larger petri dishes lined with saturated filter paper. The lids of the larger dishes are secured (try to keep the germination atmosphere at 100% RH at all times) and the boxes are kept at 20-25°C for 48 hours. Exposure to light does not seem to affect germination.

6. Germinating pollen grains are counted directly on the agar. Normally, three fields are observed using x40 or x100 magnification. Depending on the germination percentage of the lot, attempt to count a total of about 100 grains for high (>80%) or low (<20%) germination, or about 300 grains for intermediate germination (50%). Four classes of germinating pollen grains can be observed:

   Class 1 pollen tubes have elongated >2x the diameter of the original pollen grain.

   Class 2 pollen tubes have begun to elongate but are <2x pollen grain diameter.

   Class 3 not germinating; showing no evidence of pollen tube growth.

   Class 4 pollen tubes have formed but are showing irregular growth (bulging) or plasmolysis (contents of the tube are shrinking away from the tube wall).

7. In scoring the germination percentage of a pollen lot, only the class 1 and 2 pollen grains are scored. Adding class 1 and 2 grains and dividing by the total number of pollen grains observed determines the pollen lot's germination percentage. Scoring class 1 and 2 grains separately is desirable because the percentage of class 1 grains may be an indication of vigour potential. This may have more apparent significance when formulating specific pollen poly-mixes of varying viability response.

8. Estimating fertility potential of any particular pollen lot from germination results may be possible if broad categories are established. Until more definitive tests are completed, the following is proposed:

Germination >80%:	expect good yields
Germination 50-80%:	expect moderate to good yields
Germination 30-50%:	expect poorer yields
Germination <30%:	do not use or recheck

One must remember that these categories are not definite and discarding a lot based solely on its germination results may not be appropriate!

3.23 Electrical conductivity procedures

Conductivity procedures follows those reported by Ching and Ching (1976) in which 100 mg of pollen (prehydrated according to procedure 4 described in Section 3.22) is soaked in 30 mL de-ionized water

(specific conductance <2 µS/cm) at 25°C for 60 min with constant shaking. After 60 min, the pollen is permitted to settle and the conductivity of the solution determined with an immersion cell (platinum electrodes). Since conductivity is very sensitive to temperature, it is important to ensure the temperature at measurement is 25°C. All conductivity results are expressed on a dry weight basis of the pollen sample used (i.e. µS/cm/gdw).

In all experiments tested to date, % conductivity provides better estimates of fertility potential than conductivity alone. Percent conductivity is the ratio of cold leaching (described above) to hot leaching. After the cold conductivity is determined the pollen-water mix is heated to 95°C for 1 hour. The hot solution is then cooled and equilibrated at 25°C before measurement.

Several conductivity meters exist on the market and the only important criteria for selecting an instrument is its sensitivity. Most measurements are made in the µS to mS range. Also, the cell constant of the immersion probe can vary (i.e., <1 or >1). To calibrate a probe, check the conductivity of a 0.01N KCl solution. For a cell constant of 1.0, the conductivity of the solution should be 1.41 mS/cm.

Since conductivity is a measure of cell membrane integrity, the relationship between leachate conductance and fertility is an inverse one. For Douglas-fir pollen, % conductivity values above 50% were correlated to low seed yields. The relationship between conductivity and fertility in interior spruce has yet to be detailed but if a particular pollen lot yields % conductivity values greater than 50%, then it may be appropriate to question its use (do not, however, discard a pollen lot based on this recommendation alone!). Certainly it would be wise to compare the % conductivity value with the % germination response before making a decision to include or exclude a particular lot. Remember, % conductivity is inversely related to % germination (and fertility).

3.24 Field testing

Determining germination or conductivity response of a pollen lot is of little use if it can not be related to fertility potential. The classes shown in procedure 8, Section 3.22 are only a rough estimate, and research to improve the relationship between *in vitro* response and actual fertility will continue. In the initial stages of implementing operational viability assays, it is strongly recommended to include field fertility trials of *in vitro* tested lots if for no other reason than to gain confidence in interpreting assay response.

If pollen testing is done in the spring, before the pollination season, then field testing under controlled crossing conditions is possible. For each lot, place one (preferably two) pollination bags containing three or four seed-cone buds on each of four clones (more if you can afford the time) before bud burst. Make certain all the pollen-cone buds are removed before securing the bag. Pollinate the bags at their most receptive stage (see Section 3.32) with about 0.5-1 mL pollen per bag. A pollination syringe works very well for pollinations as does a small fine camel-hair brush. Adding a small cube (1 cc) of no-pest strip to each bag protects against insect damage. Various manufacturers supply no-pest strip, and they all have the active ingredient of dichlorvos (about 18%). The white pollination bags (supplied by Delta Bags of Toronto) can be left on the tree until the cones mature, but more often they are replaced with light mesh insect bags. In the fall, cones are hand extracted and the number of filled seeds per cone determined (by squashing the seed or by X-ray analyses). Section 3.22 and 3.23, provide a brief discussion of the relationship between assay response and fertility potential and offer rough limits for classifying potential fertility from *in vitro* response.

3.3 Pollen Application

Regardless of whether stored or fresh pollen is used, one of the most important factors affecting both the yield and genetic composition of production seed is the method and timing of pollen application. First, it is important to define receptivity stages. White and Engelmann spruce have identical pollination mechanisms, and for more detail, users are encouraged to review pertinent publications by Dr. J.N. Owens of the University of Victoria, and others (see Section 5 for a list of relevant references). Second, it is essential to apply pollen during the optimal receptivity period. Although seed cones are receptive to pollen for a 12- to 14-day period (this period can be considerably less in warmer conditions), maximum receptivity occurs within a considerably shorter period of time (4-6 days).

Attempting to pollinate all cones at the optimal receptivity will be very difficult. Variation in receptivity within cones, within trees and between clones occurs naturally — a mechanism the species has developed to ensure pollination can occur under a wide range of conditions. However, for operational seed orchard management, variation creates more work. Third, the manner in which pollen is applied is of less importance biologically, but it can influence operational efficiency.

3.31 Seed cone receptivity

Five phenological stages of seed-cone bud development (Figures 2A) have been defined for interior spruce. These stages follow similar developmental patterns previously defined for pollen-cone buds (see Section 3.11).

Stage 1 is the dormant bud. At this stage, distinguishing between male, female or vegetative buds without dissection is virtually impossible. Seed-cone buds are normally located in the upper regions of the crown and usually at the tips of the shoots, although considerable variation exists.

Stage 2 buds are characterized by a slow but steady increase in bud girth and length over a 4 or 5 week period. Seed-cone buds begin swelling in response to post-dormancy development of the ovuliferous scale. It is possible at the later stage of development to distinguish seed-cone buds from male and vegetative buds by their position in the crown and on the stem. Normally, seed-cone buds are more slender in appearance and usually occur on terminal positions (but in heavy crop years, seed-cone buds can occur along the stem). The only certain way to distinguish between bud types is to wait for bud burst.

As for pollen buds, this is also a sensitive stage of development, and exposure to extreme heat or cold can lead to abnormal development or death.

Stage 3 of seed cone development is bud burst (BB). About 4-6 weeks after bud swelling begins, the elongating seed-cone bud pushes through the bud scales, exposing the red or green tips of the ovuliferous scales. Bud scales can also separate in the middle of the cone, leaving the bud-scale cap on top of the bud (see Figures 2A and 2B). The cap may persist for some time and can interfere with pollination. Normally, the cap is removed before pollinations but operationally this will not be possible. Whether or not the cap's presence affects yields can be determined by monitoring its behaviour clonally.

As the seed-cone bud elongates, the ovuliferous scales become more prominent and distinguishable (see Figure 2C). This is an important identifiable stage of development to determine the beginning of the optimal receptivity stage of the seed-cone bud. About 6-8 eight days beyond bud burst (BB+6 to BB+8), the ovuliferous scales are fully reflexed and arched. The bracts, however, are less distinguishable than at BB.

Stage 4 is maximum receptivity (see Figures 2B and 2C) and is the optimal time to pollinate. Experience has shown that the time of maximum receptivity varies considerably both within the tree and within the cone. Because pollination of individual cones is not operationally feasible, stage 4 should be classified on a per tree basis. In soil-based orchards, variation in reaching stage 4 on any particular ramet can be as much as 4-8 days depending on weather, aspect (i.e., cones on the north or south side of the tree), and position in the crown. Normally a tree is rated at stage 4 when, for example, 20% of the seed-cone buds have reached stage 4. Characteristics of the maximum receptivity stages are defined in the next Section (3.32) dealing with the optimal time to pollinate interior spruce.

Stage 5 seed-cone buds are at the post-receptivity stage when ovuliferous scales have swollen to the point that pollen can no longer reach the micropylar arms. Ovuliferous scales begin to fold upright from the base of the cone. Stage 5 seed-cone buds are characterized by an upright cone with all its scales tightly packed around each other. Continued development of the seed-cone bud occurs as it turns down and matures.

3.32 Optimal time to pollinate

Two techniques for defining the optimal time to pollinate (OTP) interior spruce have been tested. In the first trial (Kalamalka clone bank), OTP was defined on the basis of the number of days beyond bud burst (BB). Figure 5 shows the seed yield values for four clones (averaged for two ramets per clone and two pollination bags per ramet). Figure 6 shows the same data but averaged over the four clones, two ramets and two pollination bags per ramet. A pollen poly-mix was used starting at BB and continuing every second day up to 14 days beyond bud burst.

When considering average values for all clones, maximum yields were obtained between BB+6 and BB+10 days (Figure 6). However, considering clones separately, the optimal time to pollinate interior spruce ranged between BB+6 to BB+10-12 days (Figure 5).

Figure 6 also shows a comparison of pre-dawn and noon pollinations. Since maximum seed yields are obtained during the period of seed cone development when pollination drops are most prevalent (i.e., BB+6 to BB+10 days), it is possible that higher seed yields might have been obtained if pollinations were completed during a period of the day when plant moisture stress was lowest (assuming that plant moisture stress affects the production of pollination drops).

Results shown in Figure 6 suggest that pre-dawn pollinations offer no advantages. Although this test was done in a moderately cool year (1987), there appears to be no reason to consider time of day as a pollination factor.

In a second OTP trial, six clones selected at the Skimikin seed orchard (1988) were pollinated according to morphological development of the seed-cone bud rather than on a set number of days beyond BB. In this test, three stages of receptivity (early, mid- and late) were defined. The early stage of receptivity occurred within the first few days after bud burst and was characterized by small ovuliferous scales and relatively larger, prominent bracts. The ovuliferous scales were still relatively flat against the stalk of the cone and were only marginally larger than the bracts (Figure 2A). The cone itself was very fragile and no thickening of the central axis had occurred.

In the mid-stage of receptivity (mid-1), the cone axis had elongated, and the ovuliferous scales had grown larger and begun to reflex open relative to the bracts. As the mid-stage continued to develop, the scales reflexed even further and began to curl (mid-2). The bracts became even less distinguishable. At the later stages of receptivity (mid-3), the scales were fully reflexed, and in many clones curling of the scale was accentuated (Figure 2C). When the cone was viewed from the side, the reflexing and curling of the scales gave the cone a honey-comb effect. It is at this latter stage of development that maximum receptivity occurred. The cone scales were fully exposed and had the greatest funnelling effect, which aided in directing pollen down the cone axis towards the micropylar arms of the ovuliferous scales (see Owens *et al.* 1987). The later stage of cone development began when scales no longer reflexed and swelling began to force the scales against the cone axis. Bud closure (BC) began at the bottom of the cone and progressed to the top.

Figures 7 and 8 show average seed yields from clones pollinated at five morphological stages of seed cone receptivity. In Figure 7, four of the six clones tested produced the highest yields when pollinated at the later stages of receptivity (mid-3). This is also the stage of development when pollination drops are most prevalent (see Owens *et al.* 1987). Figure 8 shows the yields averaged for all six clones.

Pollen was applied only once in these tests. It may be possible to produce operational quantities of seed with single pollen applications, but it would require several visits to the tree to pollinate all the cones at their optimal receptivity. Considerable variation in seed-cone bud receptivity can occur both within the tree and within the cone. It appears that maximum receptivity corresponds to the exuding of the pollination drop, which, according to Owens *et al.* (1987), appears on the lower cone scales first and moves upwards (appears acropetally). Owens *et al.* (1987) also suggest that if the drops do not receive pollen, they persist. However, once pollinated, the pollination drops recede quickly.

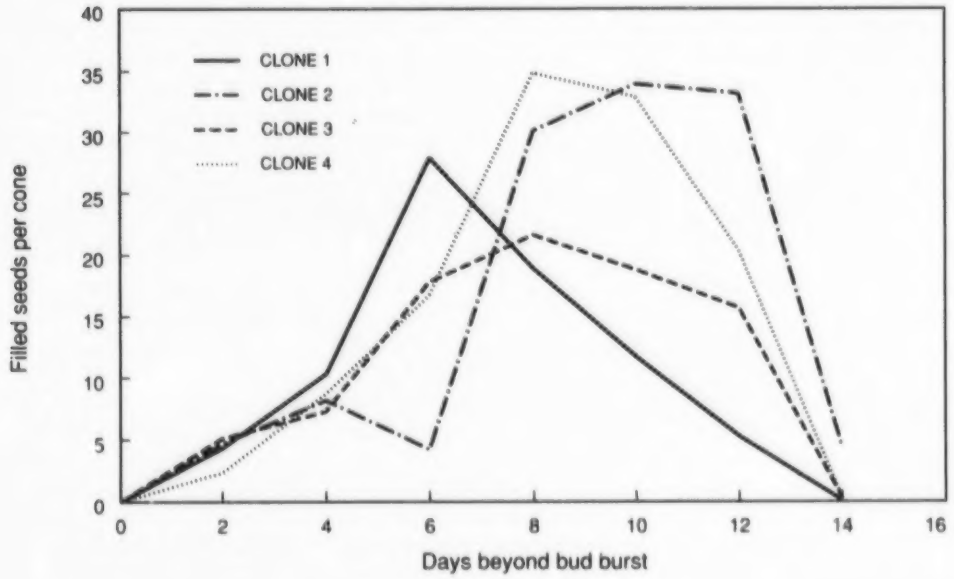


FIGURE 5. Effect on seed yield of pollinating four interior spruce clones at various numbers of days beyond bud burst.

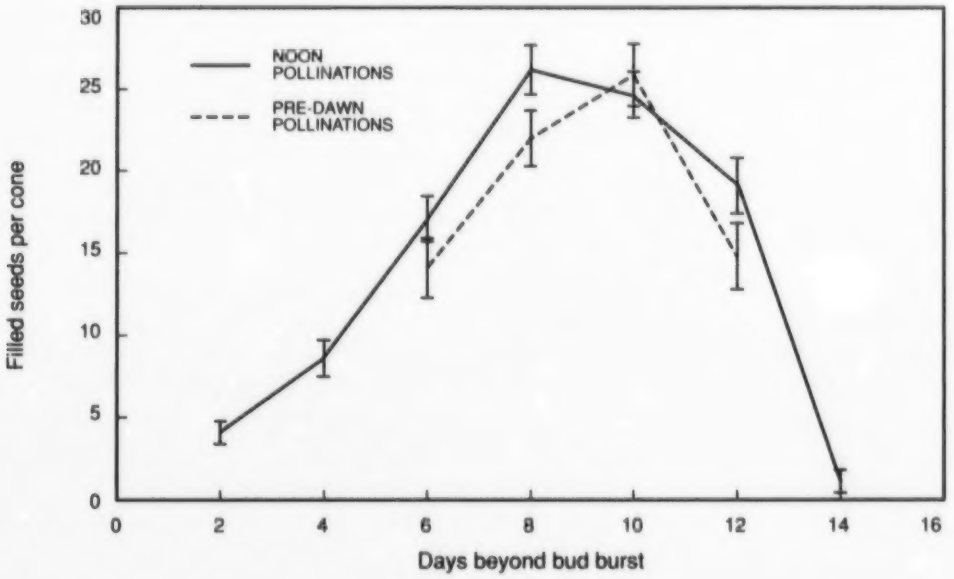


FIGURE 6. Effect on seed yields of pollinating interior spruce (clones bulked) at various numbers of days beyond bud burst, with a comparison of pre-dawn versus noon pollinations.

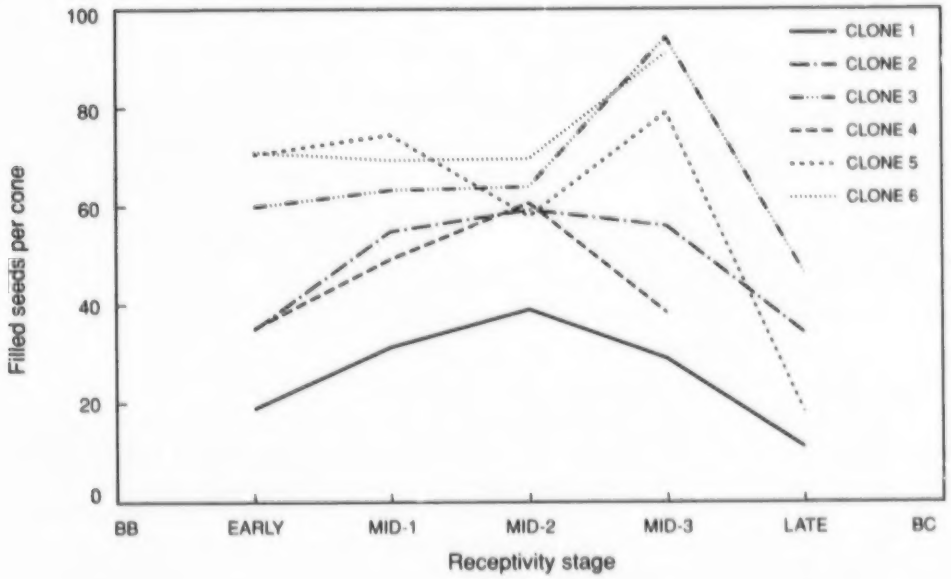


FIGURE 7. Effect on seed yields of pollinating six interior spruce clones at five post-bud burst stages of morphological development in the seed cone.

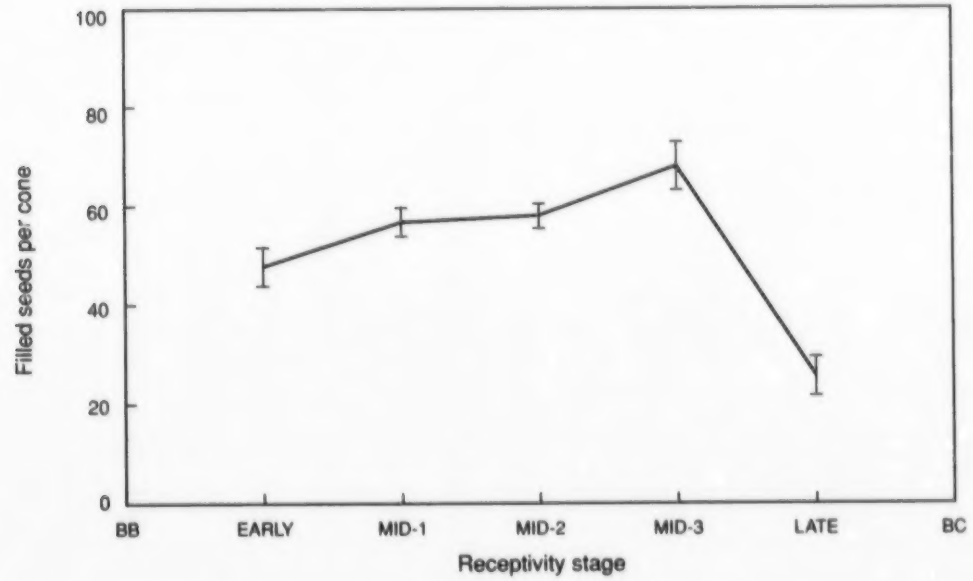


FIGURE 8. Effect on seed yield of pollinating interior spruce (clones bulked) at five post-bud burst stages of morphological development in the seed cone.

In this test, seed-cone buds were isolated and only received pollen on the date indicated. Under field conditions and ample pollen supply, pollination drops could be continually receiving pollen as they appear and, therefore, may not be as persistent as we observed for isolated cones. For operational pollination, early application may be very important. Starting at the early to mid-stage of receptivity, two or three pollinations for any particular tree are recommended.

Pollen arriving early appears to have an opportunity to participate in fertilization. However, it is not known whether an early arriving pollen grain will have a competitive advantage over pollen arriving later, during the appearance of the pollination drop. Little is known about the pollination dynamics within interior spruce especially regarding pollen competition studies. Such studies are being considered in future trials. In the mean time, practising good pollen handling technique and applying pollen in multiple applications should produce desired results.

3.33 Pollen preconditioning

The importance of rehydrating pollen before *in vitro* testing has already been discussed. In Douglas-fir, hydrating pollen at 100% RH for 16 hours significantly improved *in vitro* viability response (Charpentier and Bonnet-Masimbert 1983) but not fertility (Mellerowicz and Bonnet-Masimbert 1986).

One of the important effects of rehydration is to restore dehydrated membranes to a more stable form where they are protected from sudden changes in osmotic potential (e.g., adding dry pollen to an aqueous germination media). Since Douglas-fir has a "dry" pollination mechanism, it seems reasonable that rehydration would not affect *in vivo* fertility. Certainly, pollen grains arriving to the micropyle of Douglas-fir would have ample opportunity to rehydrate before germination. In spruce, however, the pollination mechanism involves a pollination drop ("wet" pollination) and rehydration of pollen before application may be beneficial.

Figure 9 shows the results for hydrating 12 one-year stored pollen lots for 4 hours at 25°C and 100% RH just before application. Results clearly show there is no effect on seed yield by prehydration, and pollen rehydration in preparation for field application is not necessary.

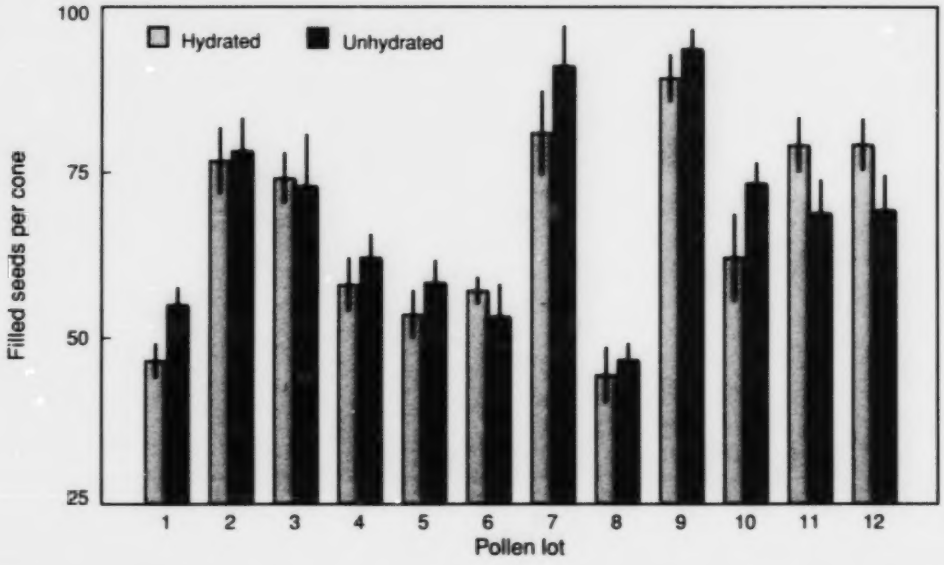


FIGURE 9. Effect on seed yield of hydrating twelve interior spruce pollen lots for 4 hours at 100% RH prior to application. Yields are shown as mean filled seeds per cone (± standard errors).

3.34 Pollen application technique

How pollen is applied will depend on the job requirements. In the breeding program, many small lots (0.2-2.0 mL) may be required to pollinate only a few flowers, whereas in the seed orchard, a few large lots (litres) of pollen may be applied to thousands of flowers.

3.341 Small lot application

Three basic pollination devices are in current use. The syringe technique employs a hypodermic needle (15 gauge) attached to a 5 or 10 mL syringe tube without the plunger. A rubber bulb is attached to the syringe end and the pollen is puffed out of the needle by hand. Pollen can be applied either directly to the flowers or indirectly (normally off the top of the pollination bag). The "power hitters" were made to handle large numbers of small applications quickly. Their operation is similar to the syringe technique but they are driven by a compressed nitrogen cylinder and have the advantage of delivering consistent amounts of pollen — an important field technique for research trials. An older but still useful pollination technique is the paint brush applicator.

Table 2 compares yields obtained from using these three pollination techniques plus a wet pollination technique. Direct application of pollen by the syringe, power hitters, and paint brush were equally effective, but the latter technique was more time consuming.

TABLE 2. Effect of three pollination devices on seed yields from four interior spruce clones (n=8).

Pollinator	Filled seeds per cone (± se)
Syringe direct	50.7 (3.7)
Syringe indirect	20.9 (2.8)
Power hitter direct	46.9 (3.2)
Power hitter indirect	23.2 (3.2)
Wet pollination aerosol	30.5 (2.3)
Paint brush	44.4 (3.4)

Indirect pollination by the syringe and power hitter applicators produced half the yields of direct application. This demonstrated that propelling the pollen between the scales and down the central axis was beneficial for optimizing seed yields in interior spruce, especially for single pollinations.

Seed yield values for wet pollination (applied as an aerosol) were actually higher than expected. Previous experience with wet pollination in Douglas-fir produced poor results, but apparently spruce was not affected. Nevertheless, wet pollinations, as an operational technique, can not be recommended because dry pollination was superior and wet pollination was more difficult to handle.

3.342 Large lot application

A supplemental pollination device was tested in the Skimikin seed orchard (1988). A portable, gas-driven compressor was designed and fabricated to deliver a consistent 30 psi to a trigger and venturi device (an Amflo Product spray cleaning gun, Model 220T). Pollen was sucked out of the reservoir and directed to the flowers through a short (0.5 m) section of aluminum pipe. No attempt was made to "blast" individual flowers but rather to spray a cluster of flowers per branch. The nozzle tip was approximately 25-30 cm from the flower cluster and about 2-4 mL of pollen was applied in a few short bursts.

Table 3 compares the seed yields from applying pollen directly with the power hitter (as described previously) to the yields form the boosting device used either once at mid-1 stage of receptivity or twice (mid-1 and again at mid-3). Flower clusters which served as controls were protected during boosting (with bags), but otherwise left exposed during their receptivity period.

Seed yields for direct pollination using the power hitter were comparable to results obtained in Table 2 and higher than comparable mid-1 stage pollinations with the boosting device. However, the boosting device was highly effective when pollen was applied once at the early stages of receptivity (mid-1) and once at the later stage (mid-3).

TABLE 3. The effect of boosting interior spruce at two stages of seed cone receptivity using controlled-crossing (power hitter direct) and boosting pollination technique (n=12).

Pollinator	Filled seeds per cone (± se)
No boosting (control)	12.4 (2.8)
Power hitter (mid-1)	47.5 (3.3)
Boosting (mid-1)	37.1 (3.6)
Boosting (mid-1 + mid-3)	52.7 (3.4)

3.4 Pollen Monitoring

A relationship between relative pollen cloud density and seed yields has been developed for Douglas-fir. Pollen was monitored at several stations within the orchard and related to seed yields from flowers exposed over a certain period of time. As orchard pollen cloud densities (grains/mm^2) increase so do yields. However, there is a point beyond which increasing pollen cloud densities are not related to further increases in seed yields. This is the 100% pollen load level and the importance of establishing this level is detailed in the protocol for rating orchard seed (see protocol for estimating SMP efficiency in Douglas-fir orchards, Webber 1991).

Pollen monitoring will be an important seed orchard management practice for estimating pollen flow within and between adjacent orchards. Contamination from surrounding stands of indigenous spruce will not be a problem for most orchards established in the south Okanagan. However, interior spruce orchard management will likely focus on equalizing parental contribution. Unequal fecundity among clones and poor pollen production, particularly in the early and late stages of orchard receptivity, will require pollen to be applied supplementally.

Developing a relationship between pollen cloud density and corresponding yields will help orchard managers estimate minimum pollen levels required to produce operational quantities on a clone or orchard basis. It will also help quantify those periods of orchard receptivity which lack sufficient pollen and may require supplemental pollination. Furthermore, if monitoring of pollen cloud density is done in the early part of orchard establishment, before its reproductive phase, then estimates of non-orchard pollen influx can be established.

Several monitoring techniques are available upon request (see Webber 1990). The easy and most commonly used procedure is to position glass slides at a 45° angle on a post within the orchard. Slides are coated with vaseline (silicone grease or epoxy resin are better), oriented in the four cardinal directions, and mounted about crown height. If the slides are replaced every day, a record of pollen density during the period that orchard trees are receptive can be obtained. Pollen grains are identified and counted for a unit area under a microscope. Normally two or three fields are surveyed and the counts are averaged and expressed as grains/mm^2.

A major disadvantage of this technique, however, is that the slides are also exposed to the weather and all the pollen can be washed off in a short rain shower (less of a problem with silicone grease). A wind-vane type monitor is very useful for long term (7-days) monitoring but does not lend itself to daily scrutiny. This device incorporates a battery driven clock covered with a slotted cap. Pollen passes through the slit and impinges on an acetate chart coated with a mixture of epoxy resin. The resin is formulated at 1 part catalyst to 27 parts resin. This ratio allows the resin to remain sticky during the recording period, but eventually (in 2 weeks) it will harden. This monitoring design has been used by the coastal Douglas-fir seed orchard group and, to a certain extent, it has been tested in Kalamalka and Skimikin seed orchards. Design features of this 7-day recorder and a brief summary of its operation are available on request (Webber and Painter 1982).

4 CONCLUSIONS

Pollen management technique can be successfully incorporated into operational seed orchard management to improve both yields and the genetic structure of the orchard crop. Although data for many procedures have been presented, testing over a number of years and conditions is still required. Data collected to date suggest that pollen can be harvested and maintained at a high vigour potential for several years. It can be applied operationally to maintain seed production goals and, most important, it allows managers several options for tailoring the genetic structure of their seed crop.

Research has made sufficient progress to recommend the incorporation of certain pollen management technique into operational use. Future research will continue to define optimal pollen extraction schedules, viability procedures, and pollen application techniques. Other areas of research will consider pollen competition studies and the effect of environmental conditions on pollen viability and vigour.

As new information and techniques become available, this information will be passed on through updates to this manual. If clarification or further elaboration of any point discussed within this manual is required, please write or call.

GOOD LUCK

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